

95. (New) A polynucleotide encoding a chimeric phosphorylation indicator, where the chimeric phosphorylation indicator comprises, in operative linkage, a donor molecule, a phosphorylatable domain, a phosphoaminoacid binding domain, and an acceptor molecule, wherein the phosphoaminoacid binding domain specifically binds to a phosphoaminoacid when present in the phosphorylatable domain, wherein the donor molecule and the acceptor molecule exhibit a detectable resonance energy transfer when the donor is excited, and wherein the phosphorylatable domain and phosphoaminoacid binding domain do not substantially emit light to excite the acceptor.

96. (New) The polynucleotide of claim 95, wherein at least one of the donor molecule or the acceptor molecule is a fluorescent protein.

B 97. (New) The polynucleotide of claim 96, wherein each of the donor molecule and the acceptor molecule is a fluorescent protein.

98. (New) The polynucleotide of claim 96, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP.

99. (New) The polynucleotide of claim 98, wherein the red fluorescent protein is a *Discosoma* RFP or a fluorescent protein related to a *Discosoma* RFP.

100. (New) The polynucleotide of claim 99, wherein the *Discosoma* RFP is DsRed or a mutant thereof.

101. (New) The polynucleotide of claim 100, wherein the DsRed comprises an amino acid sequence as set forth in SEQ ID NO:12 or a mutant of SEQ ID NO:12.

102. (New) The polynucleotide of claim 101, wherein the mutant of SEQ ID NO:12 comprises an I125R mutation.

103. (New) The polynucleotide of claim 98, wherein the fluorescent protein is a GFP selected from an *Aequorea* GFP, a *Renilla* GFP, a *Phialidium* GFP, or a fluorescent protein related to an *Aequorea* GFP, a *Renilla* GFP, and a *Phialidium* GFP.

104. (New) The polynucleotide of claim 103, wherein the fluorescent protein related to the *Aequorea* GFP is a cyan fluorescent protein (CFP), or a yellow fluorescent protein (YFP), or a spectral variant of the CFP or YFP.

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105. (New) The polynucleotide of claim 103, wherein the fluorescent protein related to the *Aequorea* GFP is an enhanced GFP (EGFP; SEQ ID NO:4), an enhanced CFP (ECFP; SEQ ID NO:6), an ECFP(1-227) (amino acids 1 to 227 of SEQ ID NO:6), an EYFP-V68L/Q69K (SEQ ID NO:10), an enhanced YFP (EYFP; SEQ ID NO:8), or citrine.

106. (New) The polynucleotide of claim 96, wherein the fluorescent protein comprises a mutation of an amino acid residue corresponding to A206, L221, F223, or a combination thereof of SEQ ID NO:2.

107. (New) The polynucleotide of claim 106, wherein the mutation corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO:2.

108. (New) The polynucleotide of claim 106, wherein the mutation corresponds to an A206K mutation, an L221K mutation, an F223R mutation, or an L221K and F223R mutation of SEQ ID NO:6 or SEQ ID NO:10.

109. (New) The polynucleotide of claim 95, wherein the donor molecule is a fluorescent protein, and the detectable resonance energy transfer is fluorescent resonance energy transfer.

110. (New) The polynucleotide of claim 95, wherein at least one of the donor molecule or the acceptor molecule is a luminescent molecule.

111. (New) The polynucleotide of claim 110, wherein the luminescent molecule comprises a lanthanide.

112. (New) The polynucleotide of claim 111, wherein the luminescent molecule comprises a terbium ion (Tb^{3+}) chelate.

113. (New) The polynucleotide of claim 112, wherein the Tb^{3+} chelate comprises Tb^{3+} and triethylenetetraaminehexaacetic acid (TTHA).

114. (New) The polynucleotide of claim 113, wherein the luminescent molecule comprises carbostyryl 124 operatively linked to the Tb^{3+} chelate.

115. (New) The polynucleotide of claim 111, wherein the luminescent molecule further comprises a cell compartmentalization domain.

116. (New) The polynucleotide of claim 115, wherein the cell compartmentalization domain is a membrane translocating domain.

117. (New) The polynucleotide of claim 116, wherein the membrane translocating domain comprises an amino acid sequence CRQIKWFNRRMKWKK (SEQ ID NO:18).

118. (New) The polynucleotide of claim 116, wherein the membrane translocating domain is operatively linked to the luminescent molecule through an amino acid sequence CCXXCC (SEQ ID NO:17).

119. (New) The polynucleotide of claim 95, wherein the donor molecule is a luminescent molecule, and the detectable resonance energy transfer is luminescent resonance energy transfer.

120. (New) The polynucleotide of claim 119, wherein the acceptor molecule is a fluorescent protein.

121. (New) The polynucleotide of claim 95, wherein the phosphorylatable domain comprises a serine/threonine kinase phosphorylatable domain.

122. (New) The polynucleotide of claim 121, wherein the serine/threonine kinase domain comprises an amino acid sequence LRRASLP (SEQ ID NO:20).

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123. (New) The polynucleotide of claim 95, wherein the phosphoaminoacid binding domain comprises an amino acid sequence of 14-3-3 τ (1-232).

124. (New) The polynucleotide of claim 121, comprising, in an orientation from the amino terminus to carboxy terminus, an ECFP(1-227) (amino acids 1-227 of SEQ ID NO:6), an MH linker, a 14-3-3 τ (1-232) phosphoaminoacid binding domain, an AGGTGGS (SEQ ID NO:19) linker, an LRRASLP (SEQ ID NO:20) phosphorylatable domain, a GGTGGSEL (SEQ ID NO:21) linker, and a citrine.

125. (New) The polynucleotide of claim 95, wherein the phosphorylatable domain comprises a tyrosine kinase phosphorylatable domain.

126. (New) The polynucleotide of claim 125, wherein the tyrosine kinase phosphorylatable domain comprises an amino acid sequence selected from EEEAEYMNMAPQS (SEQ ID NO:23) and EIYGEF (SEQ ID NO:25).

127. (New) The polynucleotide of claim 95, wherein the phosphoaminoacid binding domain comprises a Src homology domain-2.

128. (New) The polynucleotide of claim 125, comprising, in an orientation from the amino terminus to carboxy terminus, an ECFP(1-227) (amino acids 1 to 227 of SEQ ID NO:6)

molecule, an SH2 phosphoaminoacid binding domain from Shc, a GSHSGSGKP (SEQ ID NO:22) linker, a phosphorylatable domain comprising EEEAEYMNMAPQS (SEQ ID NO:23), and citrine.

129. (New) The polynucleotide of claim 125, comprising, in an orientation from the amino terminus to carboxy terminus, an ECFP(1-227) (amino acids 1 to 227 of SEQ ID NO:6), an SH2 phosphoaminoacid binding domain from c-src, GSTSGSGKPGSGEGS (SEQ ID NO:24), a phosphorylatable domain comprising EIYGEF (SEQ ID NO:25), and citrine.

130. (New) The polynucleotide of claim 95, wherein at least one amino acid of the phosphorylatable domain is phosphorylated.

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131. (New) The polynucleotide of claim 130, wherein the amino acid is serine, threonine, tyrosine, or a combination thereof.

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132. A polynucleotide encoding a chimeric phosphorylation indicator, wherein the chimeric phosphorylation indicator comprises a phosphorylatable polypeptide and a fluorescent protein.

133. (New) The polynucleotide of claim 132, wherein the phosphorylatable polypeptide comprises an N-terminal portion and a C-terminal portion, and wherein the fluorescent protein is operatively inserted between the N-terminal portion and C-terminal portion of the phosphorylatable polypeptide.

134. (New) The polynucleotide of claim 133, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP.

135. (New) The polynucleotide of claim 133, wherein the fluorescent protein is a circularly permuted fluorescent protein.

136. (New) The polynucleotide of claim 133, wherein the phosphorylatable polypeptide is a substrate for a tyrosine kinase or a serine/threonine kinase.

137. (New) The polynucleotide of claim 133, wherein the fluorescent protein is operatively inserted into a hinge region or a turn in the phosphorylatable polypeptide.

138. (New) The polynucleotide of claim 132, wherein the chimeric phosphorylation indicator further comprises a phosphoaminoacid binding domain operatively linked to the phosphorylatable polypeptide, wherein the fluorescent protein comprises an N-terminal portion and a C-terminal portion, and wherein the phosphorylatable polypeptide and operatively linked phosphoaminoacid binding domain is operatively inserted between the N-terminal portion and C-terminal portion of the fluorescent protein.

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139. (New) The polynucleotide of claim 138, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a fluorescent protein related to a GFP or an RFP.

140. (New) The polynucleotide of claim 139, wherein the fluorescent protein is an enhanced yellow fluorescent protein (EYFP).

141. (New) The polynucleotide of claim 138, wherein the phosphorylatable polypeptide and operatively linked phosphoaminoacid binding domain is operatively inserted between an amino acid sequence corresponding to amino acid positions 145 and 146 of SEQ ID NO:2.

142. (New) The polynucleotide of claim 141, wherein the fluorescent protein is an EYFP.

143. (New) The polynucleotide of claim 132, wherein at least one amino acid of the phosphorylatable polypeptide is phosphorylated.

144. (New) The polynucleotide of claim 143, wherein the amino acid is serine, threonine, tyrosine, or a combination thereof.

145. (New) The polynucleotide of claim 95, which is operatively linked to an expression control sequence.

146. (New) The polynucleotide of claim 145, wherein the expression control sequence is a transcription regulatory element, a translation regulatory element, or a combination thereof.

147. (New) The polynucleotide of claim 132, which is operatively linked to an expression control sequence.

148. (New) The polynucleotide of claim 147, wherein the expression control sequence is a transcription regulatory element, a translation regulatory element, or a combination thereof.

149. (New) A kit comprising at least one polynucleotide of claim 95.

150. (New) The kit of claim 149, comprising a plurality of polynucleotides encoding different chimeric phosphorylation indicators.

151. (New) The kit of claim 150, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise different phosphorylatable domains.

152. (New) The kit of claim 150, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise different donor molecule or acceptor molecules or both.

153. (New) A kit comprising at least one polynucleotide of claim 132.

154. (New) The kit of claim 153, comprising a plurality of polynucleotides encoding different chimeric phosphorylation indicators.

155. (New) The kit of claim 154, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise different phosphorylatable polypeptides.

156. (New) The kit of claim 154, wherein the chimeric phosphorylation indicators encoded by the polynucleotides comprise different fluorescent proteins.

157. (New) A kit comprising at least one polynucleotide of claim 138.

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158. (New) The kit of claim 157, comprising a plurality of different chimeric phosphorylation indicators.

159. (New) The kit of claim 158, wherein the chimeric phosphorylation indicators comprise different phosphorylatable polypeptides.

160. (New) The kit of claim 157, wherein the plurality of different chimeric phosphorylation indicators comprise different fluorescent proteins.

161. (New) A vector comprising the polynucleotide of claim 95.

162. (New) The vector of claim 161 which is an expression vector.

163. (New) A host cell comprising the polynucleotide of claim 95.

164. (New) A vector comprising the polynucleotide of claim 132.

165. (New) The vector of claim 164, which is an expression vector.

166. (New) A host cell comprising the polynucleotide of claim 132.